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BCCDC Laboratory Services News

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Molecular Testing

Molecular Microbiology is a growth industry. As part of its leadership mandate in this area, BCCDC is working with the **Provincial Laboratory Coordinating Office (PLCO)** on a ***BC Molecular Microbiology Network***, with stakeholders province-wide. The PLCO Microbiology Advisory Group (PROMMIC), which includes private and public sector leaders, co-chairs this project. The Team had met several times to discuss this integrated Network approach to help develop an effective model for the rational introduction of these important but often costly new tests into BC Microbiology Laboratories.

Since many of you have asked about new additions to our Molecular Core and Surveillance Program, this Newsletter focuses on a few of the many molecular tests already available at BCCDC. Since our mandate is province-wide, we are focusing on supporting smaller hospital laboratories throughout BC in molecular microbiology, most recently in the area of prevention and infection control. The BCCDC's Mr. Bruce Gamage, Laboratory Services Provincial Infection Control Consultant, assists in screening and obtaining clinical information. He also works with the Canadian Public Health Laboratory Network's (CPHLN) Infection Control and Surveillance Subcommittee and is a leader in the recently created Provincial Infection Control Network (PIC Net).

BCCDC's Laboratory Services has a "Daisy Head" model for responding to new public health or reference microbiology testing demands. In the centre of the Daisy is Molecular Core and Surveillance Program (Molecular Services), headed by Alan McNabb. His team of experts partners with the Quality Team and Biohazard Containment Team and with medical, scientific and other technical senior staff on molecular microbiology developments. A Quality Management approach, Introduction and Validation of New Laboratory Methods, is used. Urgent requests, such as responding to possible *Neisseria meningitidis* clusters of infection last year with DNA sequencing and Pulsed Field Gel Electophoresis testing, are more of a challenge but as BC's public health laboratory, our chief mission is "Transforming Surveillance and Response. Better Public Health" and extensive use of leading edge molecular technology is one way we support this vision.

Community Acquired Methicillin Resistant *Staphylococus Aureus* (CA-MRSA)

Methicillin resistant *Staphylococcus aureus* (MRSA) has become a major nosocomial-acquired infection, often associated with risk factors such as hospitalization, residence in extended care facilities, or the presence of indwelling medical devices and catheters.

There have been increasing records worldwide of serious MRSA infections in seemingly healthy individuals, including penitentiary inmates, children, team sports athletes and men who have sex with men. Some disturbing manifestations of the disease are that younger persons are often affected and there is a disproportionately high rate (compared to Hospital Acquired or H-MRSA) of severe disease caused by this new group of MRSA, (Community Aquired-MRSA or CA-MRSA).

CA-MRSA is known to cause skin and soft tissue infections, sepsis, necrotizing pneumonia or necrotizing fasciitis. There does not appear to be clonal spread. From a scientific perspective CA-MRSA has three major distinguishing features: (a) Presence of the genetic locus coding for Panton-Valentine Leukocidin (PVL), a pore forming complex which lyses host defense cells (macrophages and polymorphonuclear cells); (b) Oxacillin resistance, but general susceptibility to most non-beta lactam antibiotics; and (c) No Staphylococcal enterotoxin genes.

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BC Centre for Disease Control

BCCDC Laboratory Services is capable of detecting CA-MRSA by PVL-specific polymerase chain reaction (PCR) and Pulse Field Gel Electrophoresis (PFGE). PFGE is most often used to identify outbreaks or clusters of MRSA in facilities across the province. It is also used to identify CA-MRSA clones.

Patients infected or colonized with H-MRSA continue to be a problem. Laboratory Services has been providing diagnostic services to many hospitals across the province that do not have the capacity to test - we offer PFGE testing for investigation of strain relatedness (cluster investigation). BCCDC Laboratory Services also uses a Multiplex PCR to detect *mecA*-positive *Staphylococcus aureus.*

Several scientific projects are underway across Canada in partnership with the Public Health Agency of Canada (PHAC)'s National Microbiology Laboratory, in Manitoba, Alberta and British Columbia, to study CA-MRSA further.

Vancomycin-Resistant Enterococci (VRE)

While culture methods are most commonly used to detect VRE, an increasing number of large laboratories now use molecular detection methods.

From a scientific perspective, vancomycin resistance is mediated by six known *van* operons, *vanA* to *E* and *VanG*. These genes (with the exception of *vanC*) are found in vancomycin resistant *Enterococcus faecium* and *Enterococcus faecalis*. The *vanC* operon is found in vancomycin resistant *Enterococcus gallinarum*, *Enterococcus casseliflavus* and *Enterococcus flavescens*. These isolates exhibit low-level resistance to vancomycin but are susceptible to teicoplanin. *E. faecium* and *E. faecalis* with *vanE* and *vanG* genotypes behave similarly with respect to susceptibility to these two antibiotics. There are exceptions as to the bacterial species carriage of the *van* operons. For example both *vanA* and *vanC* genotypes have been found within individual *E. gallinarum* isolates.

Laboratories using cultures to detect VRE should be aware of the appearance of small colony variant vancomycin-resistant *Enterococcus faecium* (scvVRE). The pinpoint colonies are visible in most media at less than 24 hours of culture. These scvVRE were first identified in Toronto in late 2004. Isolates are characterized by *ddl-E. faecium* PCR and Chaperonin 60 [Cpn60] gene identification.

BCCDC Laboratory Services offers a multiplex PCR test that can concurrently identify *E. faecium* or *E. faecalis* and the *vanA* or *vanB* genotypes. *E. faecium* and *E. faecalis* isolates harboring *vanA* or *vanB* resistance-genes constitute greater than 95% of all human isolates. VRE isolates that are multiplex PCR negative are further tested by PCR for *vanC, D, E and G* and Pulsed Field Gel Electrophoresis (PFGE) for cluster or outbreak investigation of VRE isolates.

Clostridium difficile-Associated Diarrhea (CDAD)

Recent outbreaks of *Clostridium difficile* in Quebec, and now in other parts of North America, may be due to a uniquely virulent *C. difficile* strains that cause severe diarrhea and pseudomembranous colitis.

From a scientific perspective, the virulence factors elaborated by these bacterial strains are the two large Clostridial cytotoxins (LCTs), toxin A and toxin B. The genes for both toxins are located within a 19.6 kB pathogenicity locus (PaLoc) that also encodes three other proteins, TcdC, TcdD, and TcdE. TcdC and TcdD regulate negatively and positively respectively the co-expression of toxins A and B. Genetic analysis of the Canadian epidemic strain suggest a mutation in the *Tcd C* gene that results in an enhanced expression of both toxin A and B. Cell toxicity assays of supernatants from the epidemic strain and non-epidemic strains supports these genetically-based observations. The Canadian strain also appears to produce an additional putative toxin, called the binary toxin whose specific role is currently being investigated.

It has also been observed from the Quebec outbreak that a higher proportion of patients with CDAD tend to have relapses of the disease. Unpublished data show that the relapses are due to the same original infecting strains rather than due to a different *C. difficile* strain.

The BCCDC Laboratory Services is working with BCCDC Epidemiology and the new Provincial Infection Control Network (PICN) on a surveillance program to determine the prevalence of CDAD in British Columbia. BCCDC Laboratory Services also provides PFGE for investigation of possible CDAD clusters or outbreaks.

Contact for Services

If public health laboratory support for the investigation of clusters or outbreaks in institutions are required, our protocol is to first contact Mr. Bruce Gamage, Lab Services, office telephone (604) 660-6076, pager (604) 632-9394. We will work closely with all stakeholders in assisting in appropriate testing.

The West Nile Virus (WNV) in British Columbia: This Year?

Clinical Presentations

At first WNV appeared to cause only a mild, febrile illness in humans. As the number of outbreaks of WNV in humans and horses increased, cases of neurological illness began to be described. Most WNV infections in humans are asymptomatic; approximately 20 percent of infected patients have a mild febrile illness. Some WNV infected individuals may experience an influenza-like febrile illness with an abrupt onset. Symptoms include headache, sore throat, backache, myalgia, arthralgia, fatigue, rash, lymphadenopathy, and rarely acute neurological symptoms. Approximately 1 percent of WNV infected individuals develop neurological complications including encephalitis or meningoencephalitis. More recently, poliomyelitis-like syndrome, acute flaccid-paralysis, and rhabdomyolysis has been reported. Morbidity and mortality associated with the 2002 and 2003 outbreaks were greater than previously reported.

The 2004 West Nile virus (WNV) season turned out to be very mild in most of North America with only 25 human cases across Canada. This compares to 1388 human cases in 2003. It is likely the cool, wet summer slowed the westward spread of the virus; however, in the US the virus did make its way into California and Oregon. Both of these states had large outbreaks of human illness. There was no WNV activity detected in BC in 2004 despite the extensive surveillance system established to monitor for WNV in mosquitoes, birds, humans and other animals.

Laboratory Diagnosis

Diagnosis of WNV infection requires excellent communication between laboratory services and clinicians. Serological screening tests (like all serological approaches to diagnosis) are challenging. Current test methods include ELISA IgM and IgG and hemagglutination-inhibition (HI). Other tests include polymerase chain reaction (PCR) tests. Human samples are screened using serological ELISA and HI tests and confirmed by the National Microbiology Laboratory by plaque-reduction-neutralization test (PRNT), PCR and rarely, culture. An outline of WNV diagnosis approach is shown in Table 1.

Table 1– Diagnostic Tests for WNV.

Test	Use	Turnaround Time	Comments
Hemagglutination Inhibition	Screen for flavivirus	3 days	measures total antibody to flavivirus group of viruses; acute & convalescent sera required
ELISA: Serum IgM	Screen for WNV	1-2 days	commonly persists > 1 year; cross-reactivity with other flaviviruses
ELISA: CSF IgM	Screen for WNV	1-2 days	cross-reactivity; titre decreases at 40-50 days
ELISA: Serum IgG	Screen for WNV	1-2 days	Along with IgM, titres can be used to establish acute infection
Avidity Test IgG	Status of infection	1-2 days	Determine early stage of infection
Plaque Neutralization: Serum	Confirmatory for WNV	1 week	Necessary to distinguish WNV from dengue & other flavivirus
PCR: CSF, organ transplant specimens	Confirmatory for WNV	hours	Sensitivity: 50%; false positives described
Immunohistochemistry	Confirmatory: post mortem	days	Sensitive & specific